Advancing Lung Cancer Treatment Through Multi-Omics Integration and Personalized Immunotherapy

Md Shamsuddin Sultan Khan [1*], Anton Yuryev [2], John Catanzaro [3]

Abstract

Background: Lung cancer remains a major challenge in oncology due to its complex pathogenesis and heterogeneous presentation. Traditional diagnostic and therapeutic methods often fail to address the disease's biological variations. Recent advancements in Next-Generation Sequencing (NGS) technologies, including Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS), RNA sequencing (RNAseq), and proteomics, have enhanced our understanding of cancer biology. Neo7logix, LLC utilizes these technologies to develop a precision-based approach to cancer treatment. Methods: We integrated WES, RNAseq, and urine proteomics data from a lung cancer patient using Neo7logix, LLC's platform. WES identified 264 mutated genes linked to Cancer Hallmarks pathways. RNAseq analysis provided gene expression profiles, highlighting significant expression regulators and enriched pathways. Urine proteomics detected 1,772 proteins, contributing to neoantigen selection. Peptides with high binding affinity to the patient's HLA types were identified for vaccine development. Drug recommendations were based on the personalized cancer model. Results: The integration of multi-omics data revealed complex molecular alterations and identified potential neoantigens for personalized vaccine development. Drug recommendations included Endostatin, EGFR inhibitors, and Enoblituzumab, tailored to the patient's tumor profile. Conclusion: This study demonstrates that integrating advanced sequencing technologies and personalized treatment strategies can significantly enhance lung cancer therapy. Validation in clinical settings is essential to confirm the effectiveness of these personalized approaches in improving patient outcomes.

Keywords: Lung cancer, Next-Generation Sequencing (NGS), Personalized immunotherapy, Neoantigens, Proteomics

Significance | This study highlights how integrating NGS data with personalized immunotherapy can enhance precision treatment, potentially improving patient outcomes in lung cancer.

*Correspondence. Md Shamsuddin Sultan Khan , Eman Research, 81 Flushcombe Rd, Blacktown NSW 2148 Australia.

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Introduction

Lung cancer remains one of the most formidable challenges in oncology, characterized by its complex pathogenesis and heterogeneous presentation (Siegel et al., 2023; Bray et al., 2023). Traditional diagnostic and therapeutic approaches often fall short in addressing the nuanced biological variations observed in individual patients (Jemal et al., 2022; Reck et al., 2022). Recent advancements in Next-Generation Sequencing (NGS) technologies, including Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS), RNA sequencing (RNAseq), and proteomics, have revolutionized our understanding of cancer biology by offering high-resolution insights into the molecular underpinnings of the disease (Mardis, 2022; Ley et al., 2021). Neo7logix, LLC leverages these cutting-edge technologies through its proprietary platform to provide a comprehensive, precision-based approach to cancer treatment (Smith et al., 2023; Johnson et al., 2022). Neo7logix, LLC's platform integrates a wide array of NGS data, including WES, WGS, RNAseq, and proteomics, to generate a precise and personalized profile for cancer patients (Williams et al.,

Author Affiliation.
[1] Eman Research, 81 Flushcombe Rd, Blacktown NSW 2148 Australia.
[2] Elsevier, Professional services, USA
[3] Neo7logix, LLC, 8 Case Mews Gaithersburgh, MD 20878, Maryland, USA

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2022; Garcia et al., 2021). This approach facilitates the identification of unique molecular signatures and biomarkers that drive tumorigenesis and progression (Hyman et al., 2023; Tannock & Hickman, 2021). The platform's advanced algorithms create a precision mapping, ranking, and selection profile that informs the development of personalized Immuno-molecular Augmentation (PBIMA) therapeutic applications (Lee et al., 2023; Patel et al., 2022). These applications aim to activate and enhance the immune system's defenses and regulatory mechanisms to more effectively combat the cancer and address the disease process (Kim et al., 2021; Adams et al., 2022).

In the context of lung cancer, Neo7logix, LLC's platform provides a robust framework for integrating and analyzing diverse data sources (Bardelli et al., 2022; Soria et al., 2021). WES data allows for the identification of mutations in genes that may drive tumor development and progression (Reis-Filho et al., 2022; Reddy et al., 2021). RNAseq offers insights into gene expression profiles, revealing significant regulators and pathways involved in the cancerous transformation (Wang et al., 2023; Pincas et al., 2022). Proteomics data further complements these findings by identifying protein expressions and modifications that are pivotal in tumor biology (Borrebaeck, 2021; Zhang et al., 2022).

This study focuses on a patient prototype sample with lung cancer, utilizing the Neo7logix, LLC platform to illustrate the application of these integrated technologies (Pritchard et al., 2023; Yang et al., 2022). The study outlines the process of profiling, mapping, affinity ranking, and final selection for developing a personalized cancer vaccine (Li et al., 2023; Zhao et al., 2021). By analyzing WES, RNAseq, and urine proteomics data, we aim to construct a personalized cancer model that not only enhances our understanding of the disease but also informs the development of targeted therapeutic interventions (Wang et al., 2022; Lee et al., 2023).

The integration of these advanced technologies enables the identification of neoantigens—mutated proteins that serve as potential targets for personalized vaccines (Miller et al., 2022; Rosenthal et al., 2022). The process involves ranking proteins based on several criteria, including their presence in tumor and urine samples, their expression levels, and their role in cancer hallmark pathways (Schumacher et al., 2021; Goodman et al., 2022). Additionally, the platform provides recommendations for tailored drug applications, based on the identified molecular profile, to further refine and optimize treatment strategies (O'Donnell et al., 2022; Larkin et al., 2021).

This study determined the stage for a detailed exploration of the methods and results related to the personalized cancer model and therapeutic recommendations. By combining genetic, proteomic, and therapeutic insights, we aim to advance the field of precision oncology and improve patient outcomes in lung cancer treatment (Sharma et al., 2023; Kummar et al., 2022).

2. Materials and Methods

2.1 Data Integration and Analysis

Whole Exome Sequencing (WES) Data Analysis

The WES data from the patient's lung cancer tumor was analyzed to identify genetic mutations. A total of 264 mutated genes were identified and imported into Pathway Studio, a bioinformatics tool for pathway analysis. These mutations were compared with the Cancer Hallmarks pathway collection using the "Find similar pathways" option in Pathway Studio. The identified Cancer Hallmarks pathways relevant to the mutations are listed in Table 1 and Supp Tab 1.

RNA Sequencing (RNAseq) Data Analysis

RNAseq data obtained from the patient's primary tumor was processed to determine gene expression levels. The Fragments Per Kilobase Million (FPKM) values were calculated and normalized against a transcriptome profile from normal lung tissue. This normalized data was imported into Pathway Studio for sub-network enrichment analysis (SNEA) to identify statistically significant expression regulators. Supp Table 2 provides a list of these significant expression regulators.

The significant expression regulators identified through SNEA were further analyzed to calculate their activation scores. These scores were used as input for gene set enrichment analysis to identify Cancer Hallmark pathways enriched with the most active expression regulators. The enriched Cancer Hallmark pathways are listed in Supp Table 3.

Urine Proteomics Data Analysis

Urine samples were collected from the patient at three different times of the day (morning, day, and evening) to ensure comprehensive coverage of the proteome. Proteins from these urine samples were isolated using three different methods: in-gel digestion, methanol precipitation, and in-solution digestion. Each method provided complementary results, with in-gel digestion yielding the broadest variety of proteins. In total, 1,772 proteins were detected in the patient's urine.

Development of Personalized Cancer Vaccine

Cancer Hallmark Pathways Integration

The cancer hallmark pathways identified from WES and RNAseq data were integrated to form the basis of a personalized cancer model. Pathways that shared common hallmarks were merged where possible. From the 264 mutated genes identified by WES, 35 were linked to lung cancer using the Pathway Studio

Table 1. Final Selection Precision-Based Immuno-Molecular Augmentation (PBIMA®) Selection

Peptide sequence number	Protein	Sequence
1	NCAM1	ATGGVSILK
2	PTPN11	YINANIIML
3	SEMA5A	ISYKEIGLW
4	KRT5	FSASSGLGL
5	GRIN2B	ISAQTVTPI
6	DST	LSGKGFHSW
7	PTPRH	MLTNCMEAV
8	CREBBP	ISYLDIIHF
9	CSMD3	CSSVTEPRF
10	CCDC178	ITNTEGVNK
11	FLT4	GTDARTYCK
12	AVPR1B	GLDEELAKV
13	NOTCH1	EPTSESPFY
14	ERBB4	VQIAKGMIY
15	PCDH10	DSVPDTELF
16	SERPINB4	ESYDLKETF
17	MSLN	QPLPQVATL
18	TP53	HMTEVVRRY
19	CYP2E1	RFGPVFTLH

Indicators and selection criteria combines both HLA epitope prediction and ranking affinity and PBIMA® criteria in determining final therapeutic precision application. ***Green is good binding affinity, Yellow is marginal binding affinity, Red is weak to no binding affinity. Red peptides*** *were selected based upon criteria 2-6 and will be noted as self-antigens externally synthesized and introduced as foreign epitopes for specified influences on Immuno-molecular controls on receptor signaling, sensitization or blockade.*

HLA Epitope Prediction And Ranking Criteria including *Strongest Binding Affinity in one HLA Class, Strongest / Med Binding Affinity to identical peptide sequence within multiple HLA classes, Binding Affinity Averaging selection based upon top average and Structural Prediction*

PBIMA® Ranking Selection Criteria includes *HLA Affinity Ranking, Biological Pathway Ranking, Association with Specified, Cancer Risk In Population, Literature Support, Pivotal Molecular Protein to Protein Interactions and Cross Talk (PPI-CT) in Immune Augmentation and Antigen Integrity and Sequence Viability (Thermo Fisher Antigen / Synthesis Analyzer)*

knowledgebase. Of these, only 10 were directly associated with Cancer Hallmarks pathways. To further link the remaining 25 mutated proteins to lung cancer, a manual literature review was conducted using Elsevier text-mining software. This review included searching for research articles describing protein functions in various cancers when direct evidence was lacking. As a result, 20 additional mutated proteins were integrated into the personalized cancer model. New pathways were created for some proteins, such as CYP2E1, DBI, MSLN, REV3L, POLQ, ABCA3, and SEMA5A. The 20 pathways comprising the personalized cancer model are depicted in Supp Figure 1.

Neoantigen Selection

Proteins were ranked based on several criteria for neoantigen selection, including:

Mutation presence in both circulating tumor cells (CTCs) and the primary tumor.

Protein detection in patient urine through urine proteomics.

Fold change in differential expression of protein mRNA compared with normal tissue as measured by RNAseq.

The role of the protein in Cancer Hallmark pathways.

Protein expression regulatory activity as calculated by network enrichment analysis.

Number of different cancer types linked to the protein in the literature.

Number of publications linking the protein to lung cancer.

For each of the 35 mutated proteins linked to lung cancer, 17-mer amino acid sequences surrounding mutations were identified using the NCBI RefSeq database. These sequences were submitted to the Immune Epitope Database (IEDB) to determine peptides that bind to the patient's six HLA types with the highest affinity. Patient's HLA types were determined from WES data. The final peptide selection was based on the following criteria:

Strongest binding affinity in one HLA class.

Strongest/medium binding affinity to identical peptide sequences within multiple HLA classes.

Binding affinity averaging selection based on the top average.

Structural prediction.

Supp Table 4 lists the 35 mutated proteins along with their corresponding peptides with the best affinity towards the patient's MHC class I complexes.

Drug Recommendations

Based on the personalized cancer model, the following drug recommendations were made:

Endostatin: Recommended due to mutation in COL18A1, a proprotein for endogenous endostatin. The decreased production of endostatin in the patient's tumor is linked to angiogenesis activation within the tumor.

EGFR Inhibitors: Axitinib, sorafenib, sunitinib, and pazopanib were recommended due to the transactivation of the EGFR receptor in the patient's tumor, as indicated by the "Sensitivity to growth factors" pathway (Supp Figure 2).

Enoblituzumab: Suggested because of CD276 overexpression, detected in patient urine, and its role in the "Myeloid Derived Suppressor Cell in Cancer Immune Escape" pathway (Supp Figure 1).

3. Results and Discussion

The integration of WES, RNAseq, and urine proteomics data has provided a comprehensive view of the molecular alterations in the patient's lung cancer. The identification of multiple Cancer Hallmark pathways linked to the mutations and expression regulators highlights the complexity of the cancer's molecular landscape.

The development of a personalized cancer vaccine based on the neoantigen selection process is a significant advancement. By identifying high-affinity peptides that bind to the patient's HLA types, the vaccine aims to enhance the immune system's ability to recognize and target tumor-specific antigens. This approach holds promise for personalized immunotherapy in lung cancer.

Drug recommendations based on the personalized cancer model further refine the treatment strategy. Targeting pathways and proteins specific to the patient's tumor profile can potentially lead to more effective and tailored therapeutic options. The use of endostatin, EGFR inhibitors, and enoblituzumab aligns with the molecular characteristics of the patient's cancer, potentially improving clinical outcomes.

Whole exome sequencing (WES) of the patient's lung cancer tumor identified 264 mutated genes, which were mapped to Cancer Hallmarks pathways. These pathways, including those related to cell proliferation, apoptosis, and angiogenesis, are detailed in Table 1 and Supp Table 1.

RNAseq analysis provided FPKM values, normalized against normal lung tissue, identifying significant expression regulators through sub-network enrichment analysis (SNEA). Significant regulators involved in pathways such as cell cycle regulation and immune response are listed in Supp Table 2. Gene set enrichment analysis of these regulators highlighted critical pathways, including epithelial-mesenchymal transition and tumor microenvironment interactions (Supp Table 3).

Proteomic analysis of urine samples revealed 1,772 proteins, with the in-gel digestion method providing the most comprehensive profile. This data may help identify tumor-associated proteins and potential biomarkers.

The personalized cancer model combined WES and RNAseq findings, integrating 35 mutated proteins linked to lung cancer, with additional proteins identified through literature review. New pathways were created for proteins like CYP2E1, DBI, and MSLN (Supp Figure 1).

Neoantigen selection was based on mutation presence, urine proteomics, RNAseq fold change, pathway involvement, regulatory activity, and literature evidence. 17-mer peptide sequences were analyzed for binding affinity to the patient's HLA types. The selected peptides, with the highest binding affinity to MHC class I complexes, are listed in Supp Table 4.

Drug recommendations included Endostatin for decreased production in the tumor due to COL18A1 mutation, suggesting a role in angiogenesis. EGFR Inhibitors such as Axitinib, sorafenib, sunitinib, and pazopanib, targeting EGFR signaling due to its transactivation in the tumor (Supp Figure 2). Enoblituzumab For CD276 overexpression, which may enhance immune response against the tumor.

Important Revisions was made such as COL18A1 and NOTCH3 removed. NOTCH1 and SERPINB4 added they are better candidates. Col18A1 needs to be activated in order to produce endostatin a potent inhibitor of angiogenesis. ***Our rationale is to add a cytokine booster that contains human recombinant of endostatin, IL12 and TNF-alpha along with CPG-ODN*** defense activator against the tumor tissue in concert with the neoantigens selected in the Precision-Based Immuno-Molecular Augmentation (PBIMA®).

Nano-Adjuvant is required with peptide depending upon polarity and solubility of the final sequence adjuvant carriers will be determined and selected.

4. Conclusion

The integration of multi-omics data and personalized treatment strategies represents a forward-thinking approach to cancer therapy, emphasizing the importance of tailored interventions based on individual patient profiles. Further validation in clinical settings will be crucial to confirm the efficacy of these personalized strategies.

Author contributions

M.S.S.K. drafted the original manuscript. A.Y. contributed to the data analysis and interpretation. J.C. provided critical revisions and final edits. All authors reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have conflict of interest. M.S.S.K. and J.A.C. are employee of Neo7bioscince and M.S.S.K. is the director of Eman Research.

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